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Research Article

PHYTOCHEMICAL ANALYSIS OF *Aesculus indica* SEEDS EXTRACTS

SINGH HARPREET[1]* KUMAR ARVIND [2]

School of Pharmaceutical sciences, IFTM University,Lodhipur Rajput, Email: singhharpreet747@gmail.com

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ABSTRACT

Aesculus indica is an attractive tree growing to about 30 meters (100 feet) with a spread of about 12 meters (39 feet). It is hardy to -15°C (5°F), USDA zones 7-9.It is in flower from June to July, and the seeds ripen in October. Its large leaves 10–20 cm long by 2–6 cm wide are also ornamental and the mature tree forms a beautiful round canopy. *Aesculus indica* seeds were powdered and the material is subjected to continuous hot extraction process using soxhelet apparatus with different solvents in increasing order of polarity Petroleum ether, chloroform, ethanol and water. The extracts were dried and kept in aseptic condition.The dried extract were subjected to various phytochemical analysis to detect the presence of various phytoconstituents like carbohydrates,saponins,flavonoids,tannins and phenolic compounds.

Keywords: *Aesculus indica*, Phytochemical analysis,Phytoconstituents

INTRODUCTION

Natural products have proven their potential to develop new lead for pharmaceutical, nutraceutical and agrochemical. Plants, bacteria, fungus and marine natural products are the most common source to discover a biological active molecule.[1] Natural products continue to play an important role in the discovery and development of new pharmaceuticals, as clinically useful drugs, as starting materials to produce synthetic drugs, or as lead compounds from which a totally synthetic drug is designed. The importance of natural products in modern medicine has been discussed and the value of natural products in this regard can be assessed using 3 criteria

- The rate of introduction of new chemical entities of wide structural diversity, including serving as templates for semi synthetic and total synthetic modification,
- The number of diseases treated or prevented by these substances, and
- Their frequency of use in the treatment of disease.

India has one of the richest plants medical traditions in the world. There are estimated to be around 25,000 effective plant-based formulations, the ethnopharmacology and traditional system of medicine are re-emerging to offer an attractive discovery engine. Scrutiny of medical indications by source of compounds has demonstrated that natural products and related drugs are used to treat 87% of all categorized human diseases including antibacterial, anticancer, anticoagulant, antiparasitic and immunosuppressant agents. Throughout the ages, nature has supplied the basic needs to humans, not the least of which is the provision of medicines for the treatment for a wide spectrum of disease. The World Health Organization (WHO) has estimated that approximately 65% of the world's population relies on plant derived traditional medicines their primary health care. In a survey of plant derived pure compounds used as drug in countries hosting WHO- traditional Medicine Centers, 80% of 122 such compounds identified were found to be used for the same or related ethno- medical purposes and were derived from 94 plants species[2]. For a long period of time plants have been used as a natural product for maintaining human health,especially in last decade with more intensive studies for natural therapies.According to WHO [3] medicinal plants would be a better source for to obtain a variety of drugs .About 80% of individuals from developed countries use traditional medicine which has compounds derived from medicinal plants.Therefore such plants should be investigated to better understand their properties, safety and efficiency[4].

The use of plant extracts and phytochemicals both with known antimicrobial properties can be of great significance in therapeutic treatments. In the last few years a number of studies have been conducted in different countries to prove such efficiency[5,6,7,8.]

MATERIAL AND METHODS

Prelminary phytochemical screening

Extracts were subjected to preliminary phytochemical investigation for detection of Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Saponins, Reducing sugars[9].

Test for carbohydrates

Molish's test

Extracted solution of different extracts and fractions was treated with few drops of alcoholic alpha-napthol. After that 0.2 ml of concentrated H_2SO_4 was added slowly from the side of the test tube. Formation of purple to violet color ring at the junction in ethanolic extract indicates the presence of carbohydrates.

Tests for steroids

Salkowski's test

To extracts and fractions, added 2ml chloroform and few drops of concentrated H_2SO_4, shake well and allow standing for some time. Red colour is not formed in any of the extracts indicates the absence of steroids.

Test for alkaloids

Mayer's test (potassium mercuric iodide solution)

To a small quantity of different extracts and fractions, 2 ml of 5 % HCl was added and the solution was tested with Mayer's reagent for the presence of alkaloids. Non-Formation of cream color precipitate with Mayer's reagent indicates the absence of alkaloids in any of the extracts and fractions.

Dragendroff's test (potassium bismuth iodide solution)

To a small quantity of different extracts and fractions, 2 ml of 5 % HCl was added and the solution was tested with Dragendroff's reagent for the presence of alkaloids.Non-Formation of reddish brown precipitate with Dragendroff's reagent indicates the absence of alkaloids. In this observation we found that alkaloids were not present in any of the extracts and fractions.

Wagner's test (solution of iodide in potassium iodide)

A small quantity of different extracts and fractions, 2 ml of 5 % HCl were added and the solution was tested with Wagner's reagent for the presence of alkaloids. The presence of alkaloids was not observed in any of the extracts and fractions.

Hager's test (saturated solution of picric acid)

To a small quantity of different extracts and fractions, 2 ml of 5 % HCl was added and the solution was tested with Hager's reagent for the presence of alkaloids. A yellow colour precipitates is not formed with Hager's reagent which confirming the absence of alkaloids in any of the extracts and fractions.

Test for flavonoids

Lead acetate test

To small quantity of residue, add lead acetate solution, yellow coloured precipitate was formed confirming the presence of flavonoids in all the extracts .

Sodium hydroxide test

Addition of increasing amount of sodium hydroxide to the residue shows yellow colouration, which decolourises after addition of acid confirming the presence of flavanoids in all the extracts .

Test for saponins

Foam test

Few ml of extracts and fractions was taken in test tube with small amount of water and shaken vigorously for one minute and observed for formation of foam which was stable for more than five min. All four extracts shows the foam test positive.

Test for tannins and phenolic compounds

Ferric chloride test

To a small quantity of different extracts and fractions was evaporated to dryness and residue taken up in a few ml of alcohol. To these 2-3 drops of 5% ferric chloride solution was added. Development of greenish violet color in the ethanolic extract indicated the presence of tannins.

Lead acetate test

A small quantity of different extracts and fractions was evaporated to dryness and residue taken up in a few ml of alcohol. To these 2-3 drops of lead acetate solution was added. Development of white precipitate in the aqueous extracts indicated the presence of tannins.

Dilute nitric acid test

Taken a small quantity of different extracts and fractions were evaporated to dryness and residue taken up in a few ml of alcohol. To these 2-3 drops of dilute HNO_3 solution was added. Development of reddish to yellow color in the ethanolic extracts indicates the presence of tannins.

Dilute iodine solution

Transient red color was observed indicated the presence of tannins in the ethanolic extracts.

Acetic acid solution

Red color solution was observed indicated the presence of tannins in the ethanolic extracts.

Test for proteins

Biuret test

Little ml quantity of different extracts and fractions was taken into the test tube and added 4 % NaOH and few drops of 1% $CuSO_4$ solution. Non-Formation of Violet color showed the absence of protein in all the extracts.

Test for amino acids

Ninhydrin test

Small quantity of different extracts and fractions were taken and 3 drops of 5% Ninhydrin solution was added in a test tube and boiled on a water bath for 10 min and Non-formation of purple color with Ninhydrin reagent indicated the absence of amino acid.

RESULT

The result of Phytochemical analysis of *Aesculus indica* seeds in Petroleum Ether, chloroform, Ethanol and aqueous extracts are shown in the table below.The result obtained showed the presence of carbohydrates, Flavonoids, Saponins, Tannns and Phenolic compounds.Alkaloids,steroids,Proteins, amino acids were found to be absent in all the four extracts.

Table-1: Preliminary phytochemical screening of different extracts of *Aesculus indica*

Name of test	Pet.Ether	Chloroform	Ethanol	Water
Test for carbohydrates				
Molish Test	-	-	+	-
Test for steroids				
Salkowski's Test	-	-		
Test for alkaloids				
Mayer's Test	-	-	-	-
Dragendroff's Test	-	-	-	-
Wagner's Test	-	-	-	-
Hager's Test	-	-	-	-
Test for flavonoids				
Lead Acetate Test	+	+	+	+
Sodium Hydroxide Test	+	+	+	+
Test for saponins				
Foam Test	+	+	+	+
Test for tannins and phenolic compounds				
Ferric Chloride Test	-	-	+	-
Lead Acetate Test	-	-	-	+
Dil Nitric Acid Test	-	-	+	-
Dil Iodine Solution	-	-	+	-
Acetic Acid Solution	-	-	+	-
Test for proteins				

Biuret Test	-	-	-	-
Test for amino acids				
Ninhydrin Test	-	-	-	-

+ indicate the presence of constituents and ― indicate the absence of constituents

DISCUSSION

The important secondary metabolites like Carbohydrates, Flavonoids, Tannins and Phenolic compounds were present in *Aesculus indica* seeds extracts.Medicinal plants have been used as remedies for human diseases for centuries.Saponins are secondary plant metabolites that occur in a wide range of plant species. They are stored in plant cells as inactive precursors but are readily converted into biologically active antibiotics by plant enzymes in response to pathogen attack. The natural role of saponins in plants is thought to be protection against attack by pathogens and pets[10].Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity[11].

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